Caspian Journal of Internal Medicine 2026 (Spring); 17(2): 374-384
DOI: 10.22088/cjim.17.2. 374

Original Article

Potential impact of two functional variants in the *VEGF* gene on the risk and clinical characteristics of granulomatosis with polyangiitis: A case-control study

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Received: 17 Nov 2025
Revised: 14 Dec 2025
Accepted: 26 Jan 2026
Published: 10 March 2026

Abstract

Background: Granulomatosis with polyangiitis (GPA) is an autoimmune disorder that results from an interplay of genetic factors and environmental influences. We investigated the association between two polymorphisms in the *VEGF* gene, specifically rs2010963 and rs833061, and the likelihood of developing GPA.

Methods: A case-control study involving 224 participants was conducted, comprising 104 individuals diagnosed with GPA and 120 control subjects. The high-resolution melting (HRM) technique was employed for genotyping these polymorphisms.

Results: The findings revealed a significant difference in the distribution of the CC genotype and C allele for rs2010963 between the control and case groups (CC vs GG; OR: 2.687; 95% CI [1.185-6.264], *P*: 0.014; C vs G; OR: 1.628; 95% CI [1.097-2.421], *P*: 0.012). Moreover, patients with the GC + CC genotype exhibited elevated mean levels of creatinine, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP), as well as a higher incidence of alveolar hemorrhage compared to those with the GG genotype. Concerning rs833061, no association with GPA risk was identified; however, correlations were noted with certain laboratory and clinical parameters, including PR3-ANCA levels, septal perforation, alveolar hemorrhage, renal involvement, and rapidly progressive glomerulonephritis (RPGN).

Conclusion: The C allele of rs2010963 is linked to an increased risk of developing GPA and certain laboratory and clinical parameters, while the rs833061 polymorphism does not appear to be associated with GPA risk but is correlated with various laboratory and clinical indices.

Keywords: *VEGF* gene, Genotype, Granulomatosis with polyangiitis, Polymorphism.

Citation:
Pourebrahimi A, Saghaei M, Ehtesham N, et al. Potential impact of two functional variants in the VEGF gene on the risk and clinical characteristics of granulomatosis with polyangiitis: A case-control study. Caspian J Intern Med 2026; 17(2): 374-384.

Granulomatosis with polyangiitis (GPA), previously referred to as Wegener's disease, is a rare autoimmune disorder characterized by multifactorial origins. This form of vasculitis leads to inflammation and necrosis of granulomas within small blood vessels, with its underlying causes remaining largely unidentified (1). GPA is frequently linked to the presence of circulating antineutrophil cytoplasmic antibodies (ANCAs). Over the past four decades, the incidence of ANCA-associated vasculitis (AAV), which encompasses GPA, microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA), has shown a consistent upward trend globally. A systematic review published in 2022 reported a global incidence rate of AAV at approximately 17.2 cases per million person-years, with GPA exhibiting the highest prevalence among these conditions at 96.8 cases per million individuals (2). The disease predominantly impacts the ear, nose, and throat (ENT), as well as the lungs, kidneys, and respiratory system (3). Given its rapid progression, severity, and potential for causing organ damage, GPA requires a more intensive approach to care (4).

 Publisher: Babol University of Medical Sciences

Investigating the genetic predisposition to GPA and its implications for the disease's pathogenesis is crucial for improving management strategies. However, due to its rarity, which limits the sample sizes available for genetic research, and the complexity of its pathogenesis, there have been relatively few genetic studies conducted on this condition (5). GPA is categorized as an autoimmune disease due to its pathogenesis being linked to autoantibodies, such as proteinase-3. Like other autoimmune disorders, its etiology is complex and influenced by multiple factors. While the precise cause of GPA remains unclear, both environmental and genetic factors are known to contribute to its onset. Genetic influences are particularly significant in the pathogenesis of GPA, affecting the manifestation of both active and inactive disease forms, as well as the intensity and occurrence of clinical symptoms (4, 6). Environmental factors such as infections, toxins, and smoking can change gene expression and cause inflammatory responses through epigenetic modifications (7). Both HLA (e.g., *HLA-DPB1*0401*) and non-HLA (e.g., *CTLA4*, *PTPN22*, and *SERPINA1*) genes are associated with the pathogenesis of GPA (8). Recent progress in genome-wide association studies (GWAS) has pinpointed specific genetic loci that are implicated in the pathogenesis of various common autoimmune diseases, including rheumatoid arthritis (RA), type 1 diabetes mellitus (T1DM), and inflammatory bowel disease (IBD) (9). There are two primary reasons to regard these genes as potential risk alleles for GPA. Firstly, individuals with a first-degree relative diagnosed with GPA exhibit a heightened risk for developing several common autoimmune conditions, such as RA. Additionally, GPA shares several polymorphisms with certain autoimmune diseases. Consequently, it is reasonable to further explore the genetic associations of autoimmune diseases in relation to GPA (10).

Vascular endothelial growth factor (VEGF) is a significant angiogenic factor, which can induce both physiological and pathological alterations (11-13). Connections between VEGF and the development and progression of autoimmune disorders, including systemic lupus erythematosus (SLE), RA, and multiple sclerosis (MS), have been established (14). Furthermore, investigations into the relationship between serum VEGF levels and GPA have indicated a correlation between increased serum VEGF concentrations and the pathogenesis of GPA (15). GWAS have identified numerous single-nucleotide polymorphisms (SNPs) associated with autoimmune diseases, some of which are located in the regulatory regions of genes, influencing transcription factor binding and gene expression. Analyzing these SNPs can facilitate the prediction of an individual's susceptibility to specific autoimmune conditions (16).

Numerous SNPs within the *VEGF* gene that are linked to various diseases have been identified in earlier studies (17, 18). Among these, the SNP rs2010963 is located in the 5' untranslated region (5' UTR) of the *VEGF*, while another SNP, rs833061, is situated in the promoter region. Both polymorphisms can potentially influence VEGF expression at the post-transcriptional level. Given their associations with other autoimmune disorders, it is plausible to propose that these variants may be connected to the pathogenesis of GPA (19-21). This study aimed to investigate the relationship between the rs2010963 and rs833061 variants and the onset of GPA, along with its clinical manifestations. To our knowledge, this is the first research endeavor to explore the association of these two variants with GPA.

Methods

Subjects: As a case-control study, we investigated a sample of 224 Iranian participants, which included 104 individuals diagnosed with GPA and 120 healthy controls. All patients were aged 18 years or older and met the modified GPA criteria set forth by the American College of Rheumatology (ACR) (22). They were referred to Amir-Alam Hospital of Tehran, the center for GPA in Iran, from 2024 to mid-2025. Expert rheumatologists confirmed the condition based on clinical manifestations and laboratory tests. The control group was carefully selected to match the patient group in terms of age and gender distribution, with no control participants having any previous history or signs of autoimmune or autoinflammatory diseases, and all were ANCA-negative.

Data were collected through a structured questionnaire that captured demographic details such as age, gender, age at disease onset, body mass index (BMI), and family history of GPA and related conditions. Furthermore, laboratory evaluations were performed to assess various parameters, including MPO–ANCA and PR3-ANCA, along with other relevant laboratory characteristics. The research was approved by the Tehran University Research Ethics Committee (approval number: IR.TUMS.MEDICINE.REC.1402.340), and all participants provided written informed consent before the collection of their blood samples.

Genotyping: Genomic DNA from human samples was isolated using the PrimePrep Genomic DNA Isolation Kit provided by GeNetBio, Korea. The evaluation of the DNA's quality, quantity, and suitability for genotyping was performed through spectrophotometric analysis and gel

electrophoresis techniques. The genotyping of the SNPs rs2010963 and rs833061 was carried out utilizing the polymerase chain reaction high-resolution melting (PCR-HRM) method. The PCR procedure incorporated specific forward and reverse primers, as outlined in table 1, to amplify the DNA fragments containing the specified variants. The HRM analysis was conducted utilizing the HOT FIREPol EvaGreen HRM Mix (without ROX) HRM PCR kit, with the evaluation performed using the Rotor-Gene 6000™ (Corbett Research, Mortlake, New South Wales, Australia) under the following parameters: an initial denaturation of the template DNA for 5 minutes at 95 °C for the first cycle, followed by 36 cycles consisting of denaturation at 95 °C for 20 seconds, annealing at 59 °C for rs2010963 and 58 °C for rs833061 for 25 seconds, and extension at 72 °C for 20 seconds. This technique identifies polymorphisms in the PCR product through alterations in the melting curve's shape when compared to a reference sample. The melting curve is produced by a decrease in fluorescence alongside a rise in temperature from 60 °C to 95 °C at a rate of 0.1 °C/s; variations in nucleotides lead to distinct curve patterns. Eventually, we randomly selected and sent 10% (10 patients and 12 control subjects) of the samples for direct Sanger sequencing to confirm the PCR-HRM results.

Statistical analyses: Data analysis was conducted using the SPSS Statistical Package (Version 25.0, Chicago, IL, for Windows). Descriptive statistics, such as mean±standard deviation and percentage, were utilized to effectively convey the results. The demographic and laboratory data, along with the frequencies of genotypes and alleles between the two groups, were compared using the chi-square test and independent Student's t-test, respectively. Moreover, the chi-square test was employed to assess the differences in genotype and allele frequencies of VEGF between individuals diagnosed with GPA and those without the diagnosis. Additionally, logistic regression analysis was performed to calculate odds ratios (OR), 95% confidence intervals (CIs), and p-values to investigate the association between genotype and GPA. Due to the rarity of GPA and the structure of our dataset, neither a priori nor post-hoc power calculations can be reliably performed for this study. A p-value threshold of less than 0.05 was set to indicate statistical significance.

Table 1. Primer sequences for the amplification of fragments around the three polymorphisms of the VEGF.

SNP ID	Primer sequence	PCR product length (bp)	Location	Annealing temperature
rs2010963	F: GGCTTGGGGAGATTGCTCTA R: CCCCAAAAGCAGGTCACTCA	182 bp	5' UTR	59°C
rs833061	F: TCTGTGTGGGTGAGTGAG R: TATTGGAATCCTGGAGTGA	85 bp	Promoter	58°C

UTR: Untranslated region

Results

Demographic and laboratory characteristics: Table 2 presents detailed demographic and clinical characteristics of the GPA patients. There were no significant differences in mean age, sex, or BMI between the patients and the control group (P>0.05), suggesting a good match between the two groups. Among the 104 patients, 17 reported a positive family history, while none of the healthy individuals had such a history. Additionally, table 2 shows the distribution of clinical manifestations among the patients, revealing that a notable percentage (90.3%) experienced ENT involvement, followed by constitutional symptoms at 79.8%. The laboratory characteristics of the case group are summarized in table 3.

The rs2010963 polymorphism (+405 G>C): Our research demonstrated that the distribution of the rs2010963 polymorphism genotypes in both the case and control groups conformed to the Hardy-Weinberg equilibrium (HWE) (P for patient group: 0.145, P for control group: 0.231). Table 4 indicates a significant difference in the distribution of the CC genotype between the control and case groups, with rates of 13.33% and 26.92%, respectively (P: 0.014). In the recessive model, the combined frequencies of the GC and GG genotypes (GC + GG vs. CC) showed a reduced risk for GPA (P: 0.011).

Our results suggest that the C allele was significantly less common in the healthy group than in the patient group (36.67% vs. 48.56%, respectively; P: 0.012). As shown in table 5, patients with the GC + CC genotype had higher average levels of creatinine, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) compared to those with the GG genotype, with p values of 0.044, 0.002, and 0.008, respectively. Table 6 shows that while most clinical manifestations did not show statistically significant

differences between the two genotype groups, there was one notable exception: the occurrence of alveolar hemorrhage was significantly higher in the GC + CC genotype group (27.39%) compared to the GG genotype group (9.67%), with a p value of 0.046.

Table 2. Demographic and clinical characteristics of patients with granulomatosis with polyangiitis.

Variable	Patients
Total number	104
Age (mean±SD)	44.85±13.98
Gender n	
Male	36 (34.6%)
Female	68 (65.4%)
Age of onset (mean±SD)	38.87±14.53
BMI (mean±SD)	25.26±4.34
Positive family history	17 (16.3%)
Manifestations n (%)	
Cardiovascular	13 (12.5 %)
Skin	37 (35.65)
Gastrointestinal	9 (8.7%)
Peripheral neuropathy	22 (21.2%)
Mononeuritis multiplex	7 (6.7%)
CNS	21 (20.2%)
Eye	32 (30.8%)
ENT	96 (90.3%)
Sinusitis	56(53.8%)
Nose	65 (62.5%)
Septal perforation	36 (34.6%)
Ear	55 (52.9%)
Throat	26 (25%)
Oral	26 (25%)
Lung	63 (60.6%)
Alveolar haemorrhage	23 (22.1%)
Constitutional	83 (79.8%)
Malaise/ Fatigue	78 (75%)
Fever	42 (40.4%)
Anorexia/Weight loss	24(23.1%)
Arthralgia/Arthritis	45(43.3%)
Renal	41 (39.4%)
RPGN	19 (18.3%)

BMI: body mass index; CNS: central nervous system; ENT: ears, nose, and throat; RPGN: Rapidly progressive glomerulonephritis

The rs833061 polymorphism (-460 T>C): The data in Table 4 indicate no statistically significant difference in the distribution of rs833061 genotypes between patients with GPA and the control group. Our analysis of different inheritance models for the rs833061 polymorphism revealed that the genotype frequencies did not indicate any increased or decreased risk for GPA when evaluated under dominant and recessive models ($p>0.05$). Furthermore, the frequencies of genotypes observed in both the case and control groups aligned with the predictions made by HWE (P for patient group: 0.324, P for control group: 0.131). The proportion of PR3-ANCA-positive patients was significantly higher in the TT group (84.09%) compared to the TC + CC group (43.33%), with a p-value of less than 0.001 (table 5). As shown in Table 6, septal perforation was notably more prevalent in the TC+CC genotype group, affecting 45.00% of patients, whereas only 20.45% of those in the TT genotype group were affected ($P = 0.009$). Alveolar hemorrhage occurred more frequently in the TT genotype group, with 31.81% of patients affected, compared to 15.00% in the TC+CC group (P: 0.041). Additionally, renal involvement was observed more often in the TT group, affecting 52.27% of patients versus 30.00% in the TC+CC group (P: 0.022). Furthermore, rapidly progressive glomerulonephritis (RPGN) was more prevalent in the TT genotype group (27.27%) compared to the TC+CC genotype group (11.66%) (P: 0.042).

Table 3. Laboratory characteristics of patients with granulomatosis with polyangiitis.

Variable	Patients (104)
Positive MPO–ANCA n	17 (16.3%)
Positive PR3-ANCA n	63 (60.6%)
Positive RF n	24 (23.1%)
Positive 24-hour proteinuria n	34 (32.7%)
Creatinine (mg/dL)	1.27±0.96
Vit D (ng/mL)	25.26±15.32
ESR (mm/h)	46.19±34.91
CRP (mg/l)	32.51±39.13
White blood cell (10^9/L)	9.72±7.12
Hemoglobin (g/dL)	11.99±2.25
PLT (10^9/1)	285.95±106.47
BUN (mg/dL)	20.13±11.37
AST (units/L)	22.51±10.48
ALT (units/L)	27.25±18.60
ALP (units/L)	174.00±64.45

ANCA: Antineutrophil cytoplasmic antibodies; MPO–ANCA: Myeloperoxidase ANCA; PR3-ANCA: proteinase 3 ANCA; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; PLT: Platelet; BUN: Blood urea nitrogen; AST: Aspartate aminotransferase; ALT: Alanine transaminase; ALP: alkaline phosphatase.

Table 4. Association between genotypes and allele frequency of VEGF polymorphisms with granulomatosis with polyangiitis risk.

Genotype group	Patients (n = 104) n (%)	Controls (n = 120) n (%)	OR (95%CI)	*P*
rs2010963				
GG	31 (29.81%)	48 (40.00%)	Reference	---
GC	45 (43.27%)	56 (46.67%)	0.804 (0.42- 1.52)	0.543
CC	28 (26.92%)	16 (13.33%)	2.687 (1.185- 6.264)	0.014*
Dominant inheritance				
GG	31 (29.81%)	47 (37.60%)	Reference	---
GC+CC	73 (70.19%)	78 (62.40%)	1.416 (0.78- 2.57)	0.262
Recessive inheritance				
CC	28 (26.92%)	16 (13.33%)	Reference	---
GC+GG	76 (73.08%)	104 (86.67%)	0.419 (0.196- 0.866)	0.011*
Allele				
G	107 (51.44%)	152 (63.33%)	Reference	---
C	101 (48.56%)	88 (36.67%)	1.628 (1.097-2.421)	0.012*
rs833061				
TT	44 (42.31%)	43 (35.84%)	Reference	---
TC	47 (45.19%)	55 (45.83%)	0.835 (0.452-1.541)	0.561
CC	13 (12.50%)	22 (18.33%)	0.580 (0.235-1.382)	0.229
Dominant inheritance				
TT	44 (42.31%)	43 (35.84%)	Reference	---
TC+CC	60 (57.69%)	77 (64.16%)	0.762 (0.428- 1.353)	0.338
Recessive inheritance				
CC	13 (12.50%)	22 (18.33%)	Reference	---
TC+TT	91 (87.50%)	98 (81.67%)	1.568 (0.707- 3.602)	0.270
Allele				
T	135 (64.90%)	141 (58.75%)	Reference	---
C	73 (35.1%)	99 (41.25%)	0.770 (0.514- 1.150)	0.205

Table 5. Association of VEGF polymorphisms with various laboratory parameters of granulomatosis with polyangiitis.

	rs2010963		
	GG (n =31)	GC+CC (n =73)	*P*
Positive MPO–ANCA n	7 (22.58%)	10 (13.69%)	0.263
Positive PR3-ANCA n	18 (58.06%)	45 (61.64%)	0.733
Positive 24-hour proteinuria n	9 (29.03%)	25 (34.24%)	0.604
Creatinine (mg/dL)	1.05±0.529	1.37±1.086	0.044*
Vit D (ng/mL)	24.71±11.01	25.49±16.88	0.813
ESR (mm/h)	30.74±29.49	52.75±35.14	0.002*
CRP (mg/l)	18.74±30.16	38.36±41.17	0.008*

	rs833061		
	TT (n =44)	TC+CC (n =60)	
Positive MPO–ANCA n	9 (20.45%)	8 (13.33%)	0.332
Positive PR3-ANCA n	37 (84.09%)	26 (43.33%)	<0.001*
Positive 24-hour proteinuria n	15 (34.09%)	19 (31.66%)	0.795
Creatinine (mg/dL)	1.49±1.30	1.11±0.56	0.072
Vit D (ng/mL)	26.95±15.17	24.02±15.44	0.338
ESR (mm/h)	48.79±34.42	44.28±35.43	0.518
CRP (mg/l)	40.38±43.46	26.74±34.88	0.09

* *P* value < 0.05. ANCA: Antineutrophil cytoplasmic antibodies; MPO–ANCA: Myeloperoxidase ANCA; PR3-ANCA: proteinase 3 ANCA; ESR: Erythrocyte sedimentation rate; CRP:C-reactive protein

Table 6. Association of *VEGF* polymorphisms with various clinical manifestations of granulomatosis with polyangiitis.

	rs2010963		
	GG (n =31)	GC+CC (n =73)	*P*
Skin	11 (35.48%)	26 (35.61)	0.990
Peripheral neuropathy	7 (22.58%)	15 (20.54%)	0.816
CNS	4 (12.90%)	17 (23.28%)	0.228
Eye	7 (22.58%)	25 (34.24%)	0.238
ENT	30 (96.77%)	66 (90.41%)	0.265
Sinusitis	14 (45.16%)	42 (57.53%)	0.247
Nose	15 (48.38%)	50 (68.49%)	0.053
Septal perforation	8 (25.80%)	28 (38.35%)	0.219
Ear	17 (54.83%)	38 (50.05%)	0.795
Throat	10 (32.25%)	16 (21.91%)	0.235
Oral	8 (25.80%)	18 (24.65%)	0.901
Lung	16 (51.61%)	47 (64.38%)	0.223
Alveolar hemorrhage	3(9.67%)	20 (27.39%)	0.046*
Constitutional	27 (87.09%)	56 (71.23%)	0.228
Malaise/Fatigue	26 (83.87%)	52 (73.33%)	0.173
Fever	10 (32.25%)	32 (43.83%)	0.271
Anorexia/Weight loss	7 (22.58%)	17 (23.28%)	0.938
Arthralgia/Arthritis	17 (54.83%)	28 (38.35%)	0.121
Renal	9 (29.03%)	32 (43.83%)	0.158
RPGN	3 (9.67%)	16 (21.91%)	0.140

	rs833061		
	TT (n =44)	TC+CC (n =60)	*P* -value
Skin	20 (45.45%)	17 (28.33%)	0.072
Peripheral neuropathy	10 (22.72%)	12 (20.00%)	0.737
CNS	8 (18.18%)	13 (21.66%)	0.662
Eye	15 (34.09%)	17 (28.33%)	0.530
ENT	41 (93.18%)	55 (91.66%)	0.755
Sinusitis	21 (47.72%)	35 (58.33%)	0.284
Nose	25 (56.81%)	40 (66.66%)	0.305
Septal perforation	9 (20.45%)	27 (45.00%)	0.009*
Ear	20 (45.45%)	35 (58.33%)	0.194
Throat	9 (20.45%)	17 (28.33%)	0.359
Oral	8 (18.18%)	18 (30.00%)	0.169
Lung	27 (61.36%)	36 (60.00%)	0.888
Alveolar hemorrhage	14 (31.81%)	9 (15.00%)	0.041*
Constitutional	35 (79.54%)	48 (60.00%)	0.955
Malaise/Fatigue	33 (75.00%)	45 (75.00%)	0.99
Fever	16 (36.36%)	26 (43.33%)	0.474
Anorexia/Weight loss	8 (18.18%)	16 (26.66)	0.310
Arthralgia/Arthritis	17 (38.63%)	28 (46.66%)	0.414
Renal	23 (52.27%)	18 (30.00%)	0.022*
RPGN	12 (27.27%)	7 (11.66%)	0.042*

* P value < 0.05. CNS: central nervous system; ENT: ears, nose, and throat; RPGN: Rapidly progressive glomerulonephritis

Discussion

VEGF, a signaling protein that promotes angiogenesis by directly influencing endothelial cells, is implicated in the pathogenesis of GPA (23, 24). A meta-analysis by Zhan et al. revealed that circulating levels of VEGF are elevated in patients suffering from various autoimmune diseases, such as SLE, RA, systemic sclerosis (SSc), and ankylosing spondylitis (AS) (14). Furthermore, Li et al. demonstrated that serum VEGF concentrations are significantly higher in GPA patients compared to a control group, establishing a link between elevated VEGF levels and increased disease activity (15). Prior studies have identified at least 30 SNPs within various regions of the *VEGF* gene that can influence gene expression (25, 26). Among the most extensively studied SNPs are rs2010963 and rs833061, located in the 5' UTR and promoter regions, respectively. SNPs within the 5' UTR of mRNAs can significantly affect gene expression by altering mRNA stability, protein-RNA interactions, and disrupting regulatory elements (such as internal ribosome entry sites and binding sites for regulatory proteins) (27-29). Similarly, SNPs in the promoter region can modify gene expression by affecting the binding affinity of transcription factors to their respective recognition sites within the promoter (30)

Our findings revealed that the CC genotype and C allele of rs2010963 are more prevalent in patients with GPA compared to control individuals, indicating a potential association of the C allele with an increased risk of developing GPA. Additionally, analysis using the recessive inheritance model showed that having at least one G allele is linked to a reduced risk of the disease. Concerning other autoimmune and immune-mediated inflammatory

disorders, such as Behcet's disease, giant cell arteritis, Graves' disease (GD), IBD, Kawasaki disease, psoriasis, psoriatic arthritis, RA, SLE, SSc, and T1DM, a meta-analysis conducted by Che et al. found that the C allele in Caucasian populations is associated with a heightened risk of autoimmune diseases, while an opposite trend was noted in Asian populations. Furthermore, a decreased risk of GD was observed in Caucasians possessing the G allele (19). In a similar vein, another meta-analysis indicated that the C allele may serve as a protective factor for this group of diseases in Asians, whereas a contrary conclusion was reached for Caucasians (20). A subsequent meta-analysis focusing on RA and SLE found no significant association between rs2010963 and the risk of these conditions (31, 32). Notably, a study investigating AS suggested that the CC genotype of this polymorphism may confer a protective effect on patients with AS (33). In the Iranian cohort, no significant association was identified between rs2010963 and the risk of developing vitiligo, RA, or SLE (34-36).

Our findings suggest that the presence of the C allele of rs2010963 correlates with increased creatinine levels; however, there was no statistically significant difference in the occurrence of renal impairment and RPGN between patients with GG genotypes and those with GC+CC genotypes. Literature indicates that both ESR and CRP levels are typically elevated in patients suffering from GPA, particularly in cases of generalized disease. These elevated markers are indicative of inflammation and are frequently utilized as surrogate indicators of disease activity. Our research reveals that individuals with the C allele demonstrate higher ESR and CRP levels, implying that the rs2010963 polymorphism may play a role in enhancing GPA activity. Furthermore, our results show a significantly increased prevalence of alveolar hemorrhage among patients with the C allele, potentially linked to lower levels of VEGF in these individuals. VEGF is crucial for preserving vascular integrity by supporting the survival and functionality of endothelial cells. Its inhibition may lead to increased vascular fragility and a greater risk of hemorrhage, including alveolar hemorrhage (37). The connection between the C allele and alveolar hemorrhage is particularly significant, as it indicates a genetic vulnerability to severe pulmonary complications in GPA.

In contrast to the rs2010963 polymorphism, the rs833061 variant did not show a significant association with the susceptibility to GPA. Previous studies investigating the relationship between rs833061 and other autoimmune disorders, including RA, SLE, psoriasis, and GD, also found no significant correlations (35, 36, 38-40). Nevertheless, our research identified a correlation between rs833061 and specific clinical phenotypes in GPA patients. Notably, the occurrence of positive anti-PR3-ANCA was significantly elevated among individuals with the TT genotype. Anti-PR3-ANCA antibodies play a crucial role in the pathogenesis of GPA by binding to neutrophils, which activate their autoimmune response and contribute to the development of vasculitis lesions. Although PR3-ANCA serves as a reliable biomarker for GPA diagnosis, its levels do not consistently reflect disease activity. Significantly, approximately 25% of patients exhibit no correlation between PR3-ANCA levels and disease activity, indicating that not all PR3-ANCA antibodies are pathogenic (41, 42). Furthermore, our research demonstrates that the occurrence of alveolar hemorrhage was markedly greater in individuals possessing the TT genotype, implying that the C allele may confer a protective effect against this clinical condition. One investigation revealed that the T allele at the rs833061 locus correlates with increased VEGF expression levels (25). The heightened risk of alveolar hemorrhage observed in our TT genotype patients could be linked to augmented vascular permeability, potentially resulting in pulmonary hemorrhage and additional complications such as hemosiderosis and alveolar remodeling (43). Moreover, our findings revealed a greater incidence of renal impairment and RPGN among patients with the TT genotype. In essence, in addition to its protective function concerning lung involvement, the C allele significantly aids in mitigating the risk of renal impairment and RPGN. Our data suggest that patients with at least one C allele exhibited lower creatinine levels, although this finding did not achieve statistical significance (*P*: 0.072). In contrast to the previously mentioned clinical manifestations, our results indicate that the presence of the C allele in this specific SNP may heighten the risk of septal perforation in patients with GPA. Collectively, these findings suggest that the TT genotype may predispose individuals to a more severe disease phenotype, particularly impacting the renal and pulmonary systems. The lack of a relationship between rs833061 and overall disease risk, coupled with a significant association with specific clinical symptoms, highlights the intricate role of VEGF polymorphisms in shaping disease presentation rather than its initiation.

Our research presented several advantages. Firstly, it was the first study to investigate the potential effects of SNPs in one of the key regulators of the immune system, specifically VEGF, in patients with GPA. Secondly, while previous studies have explored the links between genetic variations and complications associated with AAV (44, 45), our research specifically examined the association of VEGF polymorphisms with various clinical manifestations in GPA

patients. However, this study also faced certain limitations, which mostly emanate from low sample size and a single-center design with only participants of Iranian origin. Notably, we lacked the necessary data to assess the relationship between the two SNPs and disease severity, remission rates, relapse risk, and treatment response. Additionally, it would have been beneficial to measure serum VEGF levels concurrently, as done in several prior studies (46, 47). Furthermore, laboratory parameters of controls are unavailable for baseline comparability with patients. Moreover, the observed clinical associations in our study are only exploratory in nature because of small subgroup sizes. Lastly, conducting mRNA expression analysis could provide further insights into the functional implications of these two SNPs, as suggested by some earlier research (48, 49).

It is essential to replicate our study across various populations, as genotype and allelic frequencies may vary among different ethnic groups. In light of the common pathophysiological mechanisms underlying GPA, MPA, and EGPA, we suggest that future research should explore the associations of rs2010963 and rs833061 with MPA and EGPA. Furthermore, there has been a scarcity of pharmacogenetic studies examining the influence of gene polymorphisms on the efficacy and toxicity of drugs in GPA (50, 51). Therefore, given the significance of rs2010963 in GPA, subsequent investigations should assess the correlation between this SNP and treatment responses to enhance precision medicine. In summary, our research demonstrated that the CC genotype and the C allele of the rs2010963 SNP elevate the risk of developing GPA. Furthermore, the presence of the C allele correlates with increased levels of creatinine, ESR, and CRP, as well as a greater incidence of alveolar hemorrhage among GPA patients. Conversely, rs833061 did not demonstrate a relationship with GPA risk; however, certain laboratory and clinical parameters were found to be correlated. It is highly advisable to conduct future studies of a similar nature with a larger sample size from different centers in diverse populations.

Acknowledgments

We thank all subjects who participated in this study and appreciate any support provided by the Tehran University of Medical Sciences. Also, we would like to express our heartfelt gratitude to Dr. Samira Alesaeidi, whose invaluable assistance in sample collection will always be remembered despite her untimely passing.

Funding: No funds, grants, or other support were received.

Ethics approval: The Ethics Committee of Tehran University of Medical Sciences approved this study on 2023-09-13 (approval number: IR.TUMS.MEDICINE.REC.1402.340).

Conflict of interests: The authors declare that there is no conflict of interest. All authors mentioned have approved the manuscript. Moreover, the authors have no relevant financial or non-financial interests to disclose.

Authors' contribution: Amirali Pourebrahimi: Conceptualization, Writing – review & editing, Writing – original draft. Mozhdeh Saghaei: Writing – review & editing, Methodology, Formal analysis. Naeim Ehtesham: Data curation, Writing, review & editing. Huriyeh Hashemi: Data curation, Writing, review & editing. Seyed Amirhosein Mazhari: Data curation, Methodology. Hamidreza Karbalaei-Musa: Formal analysis. Mohammad Javad Broujerdi: Writing – review & editing. Meysam Mosallaei: Supervision, Methodology, Writing – review & editing.

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